REMARKS

After entry of this amendment, claims 2-10 and 12 are pending. Claims 1 and 11 have been cancelled without prejudice or disclaimer. New claim 12 finds support in original claim 2 and in the specification at page 2, lines 22-27, and at page 4, line 26. No new matter has been added.

The specification has been amended to add headings pursuant to 37 CFR 1.77(b) and a Brief Description of the Drawings. Support for the figure description is found in the specification, for example, at page 1, lines 10-32, and page 2 lines 1-7. The abstract has been revised to recite one paragraph as reflected in the abstract of the international application. No new matter has been added.

Objections To The Specification

A brief description of the drawings and a revised abstract are included with the amendment. In light of the amendments, the objections are believed to be rendered moot.

Reconsideration and withdrawal of the objections to the specification are respectfully requested.

Rejections under 35 U.S.C. § 112

The Examiner rejected claim 11 under 35 U.S.C. 112, first and second paragraph for alleged lack of enablement, written description, and indefiniteness. Applicants respectfully disagree. However, to expedite prosecution, claim 11 has been cancelled without disclaimer or prejudice. The rejections are believed to be rendered moot. Withdrawal of the rejections is respectfully requested.

Rejections under 35 U.S.C. § 103

Claims 1-11 were rejected under 35 U.S.C. § 103 as being obvious over Adam *et al.* (hereinafter Adam). Applicants respectfully disagree and traverse the rejection.

As acknowledged by the Examiner, Adam does not teach or suggest an assay for testing a sample for the presence or absence of inhibition of the enzymatic conversion, which comprises a reaction step in the presence of a sample and a comparison step. The Examiner alleges that "the assay can be used for drug screening" referring to page 12108, end of the left column and top of the right column. However, Adam states that "[t]he further characterization of the enzymes of the deoxyxylose phosphate pathway is a prerequisite for drug screening." The characterization of the enzymes in the pathway taught by Adam is not a teaching or suggestion of the claimed assay or the method steps as claimed.

The Examiner additionally alleges that Adam teaches an assay of enzyme activity which allegedly includes "a reducing agent (NADH, FAD including flavodoxin and a flavodoxin reductase)" (see Office Action at page 5, lines2-4). Applicants respectfully disagree with the Examiner's characterization of Adam. Adam does not disclose or mention flavodoxin or a flavodoxin reductase. Rather Adams discloses the use of NADH and FAD in their reducing system. In contrast, the present reducing system for the IspH enzyme uses NAD(P)H, flavodoxin, and a flavodoxin reductase. Furthermore, the use of NADH and FAD in Adam neither discloses nor suggests the proteins flavodoxin or a flavodoxin reductase, since NADH and/or FAD can interact with many different proteins, which proteins do not require flavodoxin. Because Adam does not teach, suggest or disclose using NAD(P)H, flavodoxin, and a flavodoxin reductase in a reducing system for the IspH enzyme as required by the claims, Adam does not render the claims obvious.

Additionally, Applicants submit herewith a summary of results from an experiment comparing an assay using NADPH and FAD in a reducing system as in Adam with an assay according to claim 2 of the present application using NAD(P)H, flavodoxin, and a flavodoxin reductase (see Appendix). The results demonstrate that the assay using flavodoxin and a flavodoxin reductase, as in the present invention, shows an approximately 7,000-fold higher specific activity of 1-hydroxy-2-methyl-(E)-butenyl 4-diphosphate reductase (IspH) than in the absence of the reductase and flavodoxin. Thus, by identifying an efficient reducing system for IspH, the present invention provides an efficient assay for testing a sample for the presence or

absence of inhibition of the enzymatic conversion of 1-hydroxy-2-methyl-(E)-butenyl 4-diphosphate into isopentenyl diphosphate and/or dimethylallyl diphosphate. The efficiency of the present assay with its high specific activity of IspH provides for applicability to large scale or industrial screening such as high-throughput screening, in contrast to the assay as in Adam with its low IspH activity.

In summary, the reference cited by the Examiner does not teach, suggest, or even mention using flavodoxin and a flavodoxin reductase. Additionally, the reference cited by the Examiner does not teach or suggest that using NAD(P)H, flavodoxin, and a flavodoxin reductase rather than NADH and FAD in a reducing system for IspH can result in a surprisingly high specific activity of IspH as evidenced by the enclosed results.

For these reasons, reconsideration and withdrawal of the obviousness rejection is respectfully requested for claim 2 and the claims dependent therefrom. *See In re Fine*, 837 F.2d 1071, 1076 (Fed. Cir. 1988) (holding that if an independent claim is nonobvious then any claim dependent therefrom is nonobvious). For these same reasons, claim 12 is likewise nonobvious.

CONCLUSION

For at least the above reasons, Applicants respectfully request reconsideration and withdrawal of the rejections and allowance of the claims.

The data summary attached in the Appendix can be verified (in a declaration) if necessary.

This response is filed within the three-month period for response from the mailing of the Office Communication, to and including December 17, 2007. No fee is believed due.

However, if a fee is due, the Director is hereby authorized to charge our Deposit Account No. 03-2775, under Order No. 13555-00001-US from which the undersigned is authorized to draw.

Respectfully submitted,

By 

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Appendix

Comparative Assay Experiment

Experimental Procedure:

Assay mixtures were prepared under anaerobic conditions in a glove box and contained 100 mM Tris hydrochloride, pH 8.0, 30 mM sodium fluoride, 2 mM NADPH, 2.3 μM [1-^{3}H]-6 (4.2 mCi μmol^{-1}), reducing agent(s), and IspH protein in a volume of 160 μl. The mixtures were incubated at 37°C. The reaction was terminated by the addition of 5 μl of 3 M trichloroacetic acid, and the mixtures were centrifuged. The supernatants were neutralized with 5 μl of 3 M sodium hydroxide, subjected to ultrafiltration (Nanosep 100 kDa membrane) and analyzed by reverse-phase ion pair HPLC as described below.

In method A, the reducing agent is 100 μM FAD.
In method B, the reducing agents are 40 μM flavodoxin and 12 μM flavodoxin reductase.

Analysis of IspH assay mixtures by reverse-phase ion pair HPLC:

Aliquots (50 μl) of the obtained mixtures described above were analyzed by reverse-phase ion pair HPLC using a Luna C8 column (5 μm, 4.6 x 250 mm, Phenomenex, Aschaffenburg, Germany), which was washed with 10 ml of 3.5% (v/v) methanol in 10 mM tetra-n-butylammonium phosphate, pH 6.0 and then was developed with consecutive gradients of 3.5 – 21 % (v/v) methanol (2ml), 21 - 35% (v/v) methanol (13 ml) and 35 - 49% (v/v) methanol (10 ml) containing 10mM tetra-n-butylammonium phosphate, pH 6.0 (flow rate, 1 ml min^{-1}). The effluent was monitored by realtime liquid scintillation analysis (Beta-RAM, Biostep GmbH, Jahnsdorf, Germany).

Results:

The specific activity of IspH protein determined by method A using FAD, as in Adam, was 177 pmol min^{-1} mg^{-1}. In contrast, the specific activity of IspH protein determined by method B using flavodoxin and flavodoxin reductase rather that FAD was 1.29 μmol min^{-1} mg^{-1}.

Thus, the replacement of FAD, as used in Adam, by a mixture of flavodoxin and flavodoxin reductase, as in the present application, increases the specific activity of IspH protein by a factor of more than 7,000.